

Stephen A. Bent
Foley & Lardner
Atty. Dkt. No. 054270/0126
Serial No. 09/508,377
Zhongyi LI, et al.
"Regulation of Gene Expression in Plants"
Sheet 1 of 39

<u>λ</u> Ε7	E7.
Bam H1 FRAGMENTS <u>E7.18</u> <u>E7.8</u> <u>E7.31</u>	E7.14
Eco R1 FRAGMENTS	
EXON-CONTAINING REGIONS ( )	 
λΕ1 Bam H1 FRAGMENTS E1.1 E1.2	E1.5
E1.3 E1.4 E1.4 E2.7	
EXON-CONTAINING REGIONS( )	
5' 3'	1 kb

FIG. 3



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RSBEI MSBEI D4cDNA PESBEII POSBE	1	.****v*p** .****ap*c	**tplp***r **sl***p	lp*******  **h***aa*  **pa****g*	pg****** **s*					
D2cDNA Consensus	meinfkvlsk	.******11 -MLCLTSSSS	prp*a* SP-S-APPR-	kic*psqh*t ***1* SRS-ADRPSP	******ggk GIIAGGGNVR					
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	1**l**qc  wd*s*t*k rlsv*p***f	ka***gv***	****ataa*v  *****p*s*mt h*saisa*lt  ***sf*s***	rg**ia**	g****** *g*gd*** **f*nig* ***kt*nigl tgygs****					
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	101 ************* ********************	******** ********* ****k**** l***h*** KDHFRYRMKR	*****gs**e ******s*** **h**k***e *v***m***	***s*****	150 ********  *******  ******  *****  *****					
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	151 *g****** *dg****** nd****** *dgis**** *gci***** hg*s*****ATVYREWA	******** ********* ********* ********	*******ak* ***d***a** ********1 ***g***** ***g****** DFNNWNGSNH	m****q**** **a**n****	200 **k*****  **k*d**k**  *******  **q*pdad*n  ****pd*ds*  *******  SIRISHVNGK					
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	201 ******* ****** ****** ****** ******* ****	***!*.g*** ***hr*d*!* ***k*sd*** ***k*n*** ***r*.h***	***********	******** **f****** ****ptr*a* **a**t**a*	***** <del>y</del> **** **** <u>1</u> *****					
RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus	251 ac******* a****t*** sg******* 1****q**** p****h**y* s*****n** -SERYVFKHP	******** **s**a**** **r****** ******** ********* RPPKPDAPRI	******** ********* ******** ********	******** k*a****** r******* **r*ns*** **r*ns**** kl*ag**** EPEVSTYREF	300 *******  ******  **d****  **d****  p****cl**  ADNVLPRIRA					



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5/44 RSBEI MSBEI D4cDNA PESBEII POSBE D2cDNA Consensus NNYNTVQLMA IMEHSYYASF GYHVTN-FFA VSSRSGTPED LKYL-DKAHS RSBEI MSBEI \*\*\*\*\*\*\* \*\*\*\*S\*m\*\* \*\*\*\*\*\*\*n \*\*\*\*\*\*t\*\* \*\*\*\*\*\*\*\* D4cDNA \*\*\*n\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\*\* S\*q\*\*\*a\*\* \*\*\*\*\*\*\* PESBEII POSBE D2cDNA Consensus LGLRVLMDVV HSHASNNVTD GLNGYDVGQS TQESYFH-GD RGYHKLWDŠR RSBEI MSBET D4cDNA PESBEII \*\*\*\*\*\*ks. s\*\*\*\*\*\* \*\*\*k\*\*\* \*\*\*\*\*\* \*\*\*\*\* POSBE \*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*V\*\*\*\*\*\* \*n \*n\*\*\*\*s\*n\* D2cDNA LFNYANWEVL RFLLSNLRYW -DEFMFDGFR FDGVTSMLYH HHGINMGFTG Consensus 451 500 RSBEI MSBEI D4cDNA d\*n\*\*\*\*e\*\* \*\*\*\*\*\*\* \*\*s\*v\*di\*\* \*\*\*d\*\*\*\*\* \*\*\*q\*q\*\*\*s PESBEII \*\*n\*\*\*\*ea\* \*\*\*\*\*\*\*\* \*\*n\*i\*\*i\*\* \*\*\*\*\*\*\* \*\*\*q\*q\*\*\*s POSBE \*\*\*\*\*iq\*\*\* n\*\*\*f\*\*\*\* \*\*\*\*\*\*l\*\* \*\*i\*\*\*<sub>V</sub>\*\*\* \*\*\*\*\*\*\* D2cDNA NYKEYFŠLDT DVDAVVYMML ANHLMHK-LP EATVVAEDVS GMPVLCRPVD Consensus RSBEI \*\*\*\*\*\* \*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*q\*.\*ah\*\* \*\*\*\*\*\*\* MSBEI D4cDNA \*V\*\*\*\*\*\* \*\*\*\*\*k\*\*\* \*\*\*\*\*k\*\*\* \*\*k\*.\*sln\* \*\*\*\*\*\*\* PESBEII POSBE \*\*\*1\*\*\*\*\*q \*\*t\*\*\*\*\*\* \*\*e\*\*q\*qq\* \*\*\*sv\*sq\*\* \*\*\*\*p\*\*f\* D2cDNA EGGVGFDYRL AMAIPDRWID YLKNKDDSEW SMSE-I--TL TNRRYTEKCI Consensus RSBEI MSBEI D4cDNA S\*\*\*\*\*\* \*\*\* \*\*\*\*\* \*\*\*\*\* \*\*e\*\*\*SS\*\* C\*tml\*\*\*\* \*\*\*S\*h\*\*\*\* PESBEII \*\*\*\*\*\* \*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* C\*td\*\*\*V\*\* \*\*\*\*h\*\*\*\* POSBE \*\*\*\*rqnh\*\* \*\*s\*\*m\*\*\* \*\*w\*t\*s\*\*\* a\*d\*d\*\*\*\* \*a\*\*\*\*\*\* D2cDNA Consensus AYAESHDQSI VGDKTIAFLL MDKEMY-GMS DLOPASPTID RGIALOKMIH

FIG. 4B



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6/44 \*\*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\* RSBEI \*\*\*\*\*\*\* \*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\* MSBEI D4cDNA \*\*\*\*\*\* \*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*q\*\*\*\*\* lt\*\*n\*\*\*n PESBEII \*f\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* .\*\*\*n\*a\*s\* POSBE D2cDNA FITMALGGDG YLNFMGNEFG HPEWIDFPRE GNNWSYDKCR -RQWSLVDTD Consensus RSBEI \*\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* MSBEI D4cDNA \*\*\*\*\*\*\* \*r\*\*1\*\*\* \*\*i\*a\*t\*\* \*\*st\*n\*\*\* \*\*\*\*\*\*\* PESBEII POSBE .....\*\*\*\* v\*\*vdtps\*\* c\*\*\*\*\*\*n\*t a\*h\*\*\*\*\*g sa\*tk\*.... D2cDNA HLRYKYMNAF DQAMNALD-K FSFLSSSKQI VSDMNEE-KV IVFERGDLVF Consensus \*\*\*\*\*\*\* K\*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* RSBEI \*\*\*\*\*K\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\*\* \*\*\*\*\*\* \*\*\*\*\*\* MSBEI \*\*\*\*\*s\*\*\* \*\*\*\*\*\*\* \*\*\*k\*\*\*\*\* \*\*m\*\*\*\*\*\* agvn\*\*\*\*\* D4cDNA \*\*\*\*\*en\*\* \*\*\*\*\*\*\* \*\*\*\*\*\* \*te\*\*\*\*\* \*\*\*\*a\*q\*\*\*\* PESBEII POSBE .\*thlrsgc\* \*p.....s\*\* stssc\*\*... .\*gpsnqspf skpfig\*pgc D2cDNA VFNFHP-KTY EĞYKVGCDLP GKYRVALDSD AL-FGGHGRV GHDVDHFTSP Consensus 751 RSBEI MSBEI D4cDNA \*\*\*\*\*\* \*\*\* \*\*\*\* .... ..... .... .... \*\*\*\* \*\*\*h\*\*\*v\* PESBEII POSBE \*\*\*\*\*\* \*\*g\*qipskc cllrehvwli telmnacq\*l kitrq\*f\*vs ifcc\*lfkge \*..... .... ..... ..... ..... ..... EG-PGVPETN FNNRP---- -----NSFKV LSPPRTCVAY D2cDNA Consensus RSBEI \*...\*\*\*\*ag agr\*lhak\*e t\*\*\*s\*\*es\* \*\*k\*s\*.... ..a....ssk MSBEI D4cDNA \*...\*\*\*kā \*kpkde\*\*\*\* w\*\*aa\*g.\*\* \*\*e\*\*\*vkda ad\*\*at\*\*sk \*...\*\*\*\*q \*\*snnpnlq\* \*ee\*\*a\*adt \*\*aripdvs\* e\*..ed\*nld PESBEII POSBE \*yqqp\*sr\*v trnlkirylq \*sv\*\*tna\*q klkf\*\*qtf\* v\*yyqqpilr D2cDNA Y---RVDER- EE-R--GAAS -GKT-PA-YI DV-ATR---- -SGE--SG--Consensus RSBEI kg\*\*\*d\*cg\* \*\*mk\*\*\*r\*\* \*e\*c\*d edk\*atagg\* \*\*wk\*argp\* \*g\*t\*\* MSBEI ka\*tqq\*ss\* \*\*in\*\*\*q\*p \*k\*n\*. D4cDNA PESBEII r\*e\*ns\*\*av dagi\*kvere vvgdn\* POSBE r\*tr\*lk\*sl stnist\*... ...... D2cDNA --SEK-DD-K KG--FVF-SS D-D-K-Consensus

FIG. 4C



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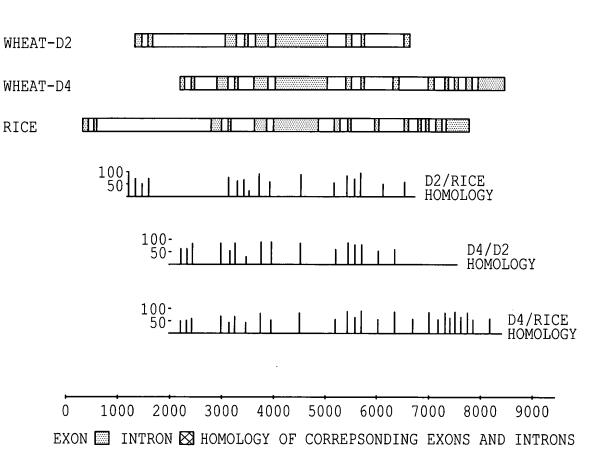


FIG. 5

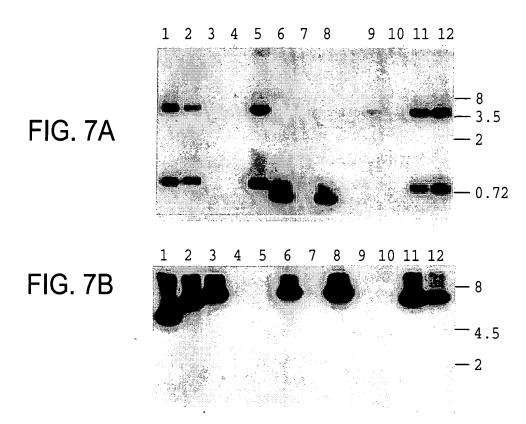


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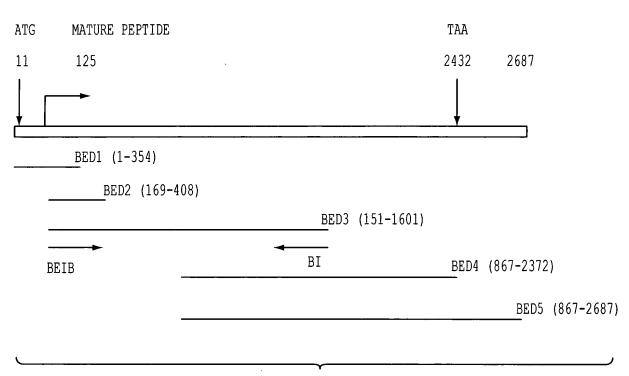


FIG. 8



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### EXPRESSION OF STARCH BIOSYNTHETIC GENES

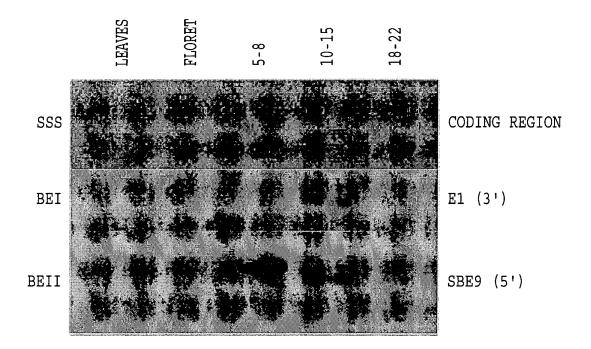


FIG. 9A



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4 6 8 10 12 15 18 21 25 31

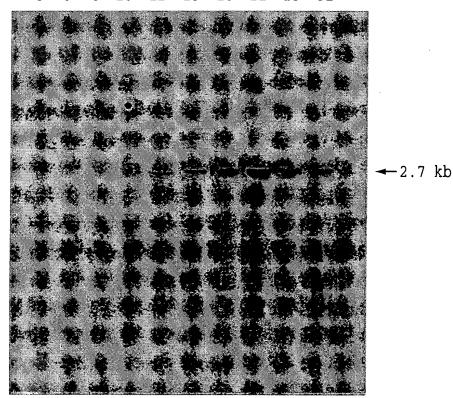


FIG. 9B



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### 4 6 8 10 12 15 18 21 25 31

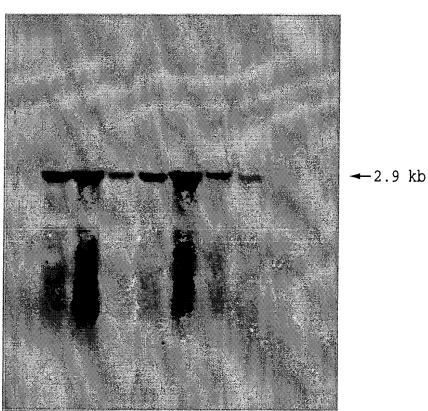


FIG. 9C



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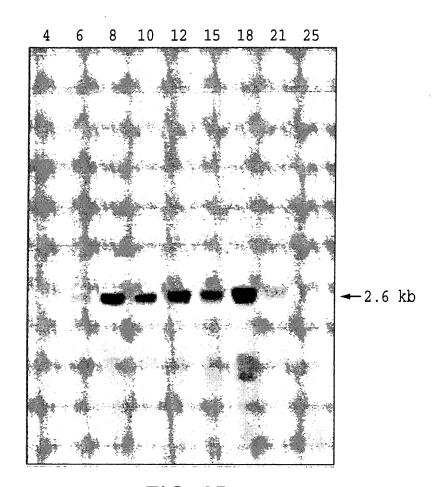


FIG. 9D



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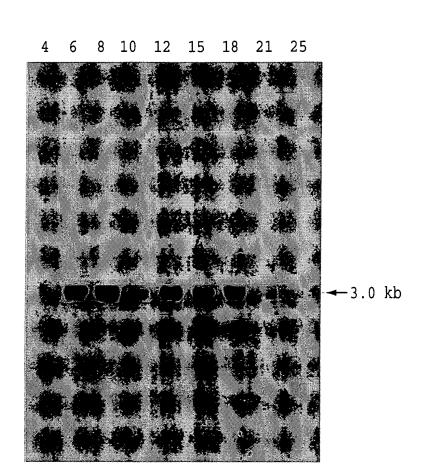


FIG. 9E



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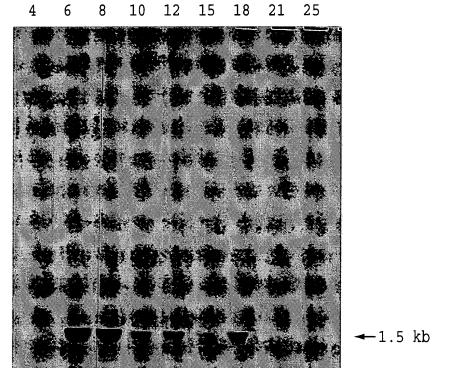


FIG. 9F



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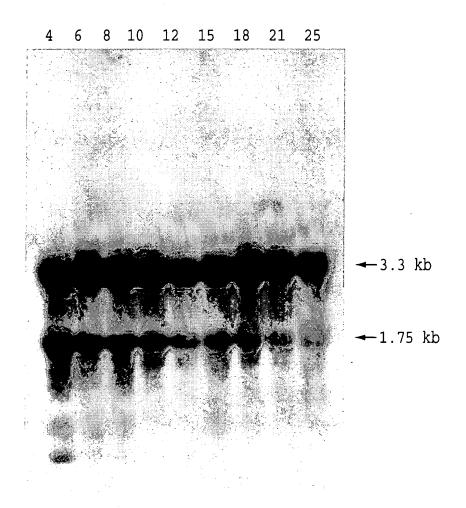


FIG. 9G



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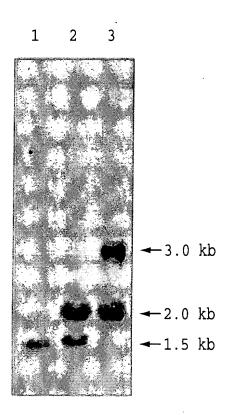


FIG. 9H



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DOTPLOT OF: d10838.pat DENSITY: 12614.77 FEBRUARY 18, 1997 11:43 COMPARE WINDOW: 21 STRINGENCY: 14.0 POINTS: 20,788

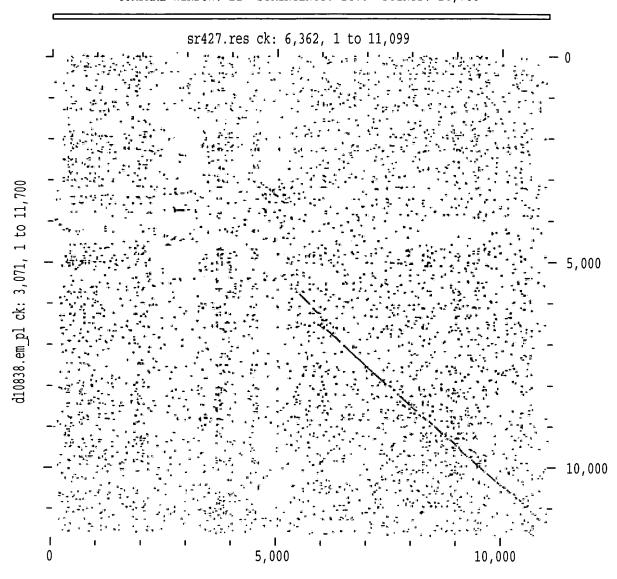
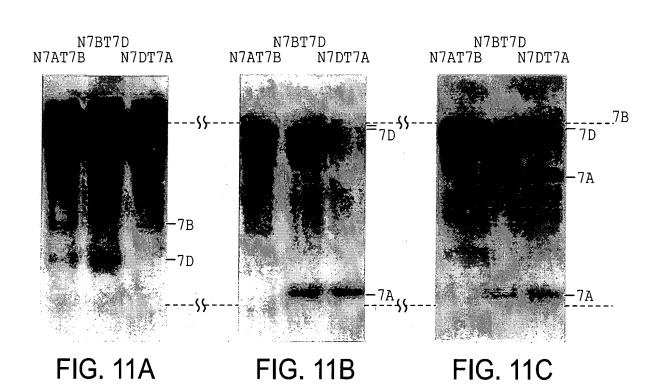


FIG. 10



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# GENOMIC CLONES FROM T.tauschii FOR SBE II.

 ${\tt BamHI}$ 

EcoRI

#### F1 F2 F3 F4 F1 F2 F3 F4

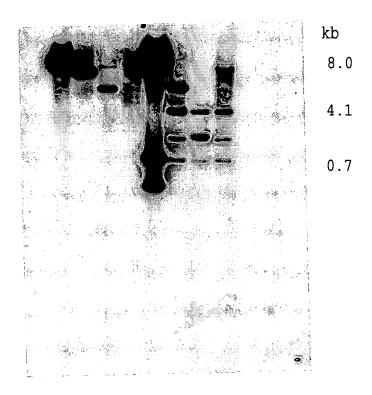


FIG. 12



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#### N-TERMINAL SEQUENCES OF CEREAL STARCH BRANCHING ENZYMES

PROTEIN	1 A	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2	2 1	2 2
RICEBEI <sup>B</sup> WBE-I <sub>AD</sub> MAIZE BEI <sup>C</sup>	A V A	T S T	A A V	R P Q	K R E	N D D	K Y K	T T	M M M	V A A	T T T	V <b>A</b> <b>A</b>	V E K	E D G	E G D	V V						
RICEBEII D	A	A	G	A	S	G	E	-	٧	M	Ι	P	Е	G	E	S	D	G	M	P	V	S
WBE-II MAIZE BEIIB	Α	A A	A A	S A	P A	<b>G</b> R	K K	- А	V V	L M	V V	P P	D E	G G	E E	S N	D D	D G	L	A A	S S	Y

A N-TERMINAL AMINO ACID OF THE MATURE POLYPEPTIDE.

RESIDUES IN THE WHEAT SEQUENCES SHOWING IDENTITY WITH THE RESPECTIVE MAIZE OR RICE BRANCHING ENZYME ISOFORMS ARE HIGHLIGHTED IN BOLD TEXT.

FIG. 13A

B KAWASAKI et al. (1993),

C BABA et al. (1991),

D MIZUNO et al. (1993),

E FISHER et al. (1993)



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TTCCCTTTTTTTTTTTTGGGNGGGGGATGCCTGTTGGATGNTGTTCCCCAATGAATTT AAGGGAAAAAAAAAAAACCCNCCCCCTACCGGACAACCTACNACAAGGGGTTACTTAAA FPFFFG?GMACWM?FPNEF а b L F F S L G G G W P V G ? C S P M N F С PFFLW?GDGLLD?VPQ\*IS-CCATGGAGTGAGAGATAGTTGGATNAGGGATCGCGNTTCCNGGAACTGTATTTTTTC GGTACCTCACTCTCTATCAACCTANTCCCTAGCGCNAAGGNCCTTGACATAAAAAAAG PWSERDSW?RDR?S?NC H G V R E I V G ? G I A ? P G T V F F S b С ME\*ER\*LD?GSRF?ELYFFP-CCCNGCGGGGGAAATGGCGTTAGTGTCNACCCAGGCCCTGGTGTTACCACGGCTTTGATC 121 -----+---+----+ 180 GGGNCGCCCCTTTACCGCAATCACAGNTGGGTCCGGGACCACAATGGTGCCGAAACTAG ? G G N G V S V ? P G P G V T T A L I а PAGEMALVSTQALVLPRL\*S-?RGKWR\*C?PRPWCYHGFDHb C ATTCTTCGTTTCATTCTGATATATTTTTCTCATTCTTTTTTCTTCCTGTTCTTGCTGTAA TAAGAAGCAAAGTAAGACTATATATAAAAGAGTAAGAAAAAGAAGGACAAGAACGACATT I L R F I L I Y I F S F F F F L F L L \*
F F V S F \* Y I F S H S F S S C S C C N a b С S S F H S D I Y F L I L F L P V L A V T - $\tt CTGCAAGTTGTGGCGTTTTTTCACTATTGTAGTCATCCTTGCATTTTGCAGGCGCCGTCC$ 241 -----+ 300 GACGTTCAACACCGCAAAAAAGTGATAACATCAGTAGGAACGTAAAACGTCCGCGGCAGG Q V V A F F H Y C S H P C I L Q A P S - C K L W R F F T I V V I L A F C R R P - A S C G V F S L L \* S S L H F A G A V L а b C TGAGCCGCGCGCCTCTCCAGGGAAGGTCCTGGTGCCTGACGGCGAGAGNGACGACTTGG ----+ 360 301 -- ${\tt ACTCGGCGCGCGGAGAGGTCCCTTCCAGGACCACGGACTGCCGCTCTCNCTGCTGAACC}$ AARPLQGRSWCLTAR?T b EPRGLSREGPGA\*RRE?RLG C S R A A S P G K V L V P D G E ? D D L A -CAAGTCCGGCGCAACCTGAAGAATTACAGGTACACACACTCGTGCCGGTAAATCTTCATA 361 -----+---+ 420 GTTCAGGCCGCGTTGGACTTCTTAATGTCCATGTGTGTGAGCACGGCCATTTAGAAGTAT V R R N L K N Y R Y T H S C R \* I F I K S G A T \* R I T G T H T R A G K S S Y b С S P A Q P E E L Q V H T L V P V N L H T-421 ------+----+ 480 а S L F T Y Q M P D E T N H G C V R b NRYSLTKCRMKPTTDASGFE-

FIG. 13B

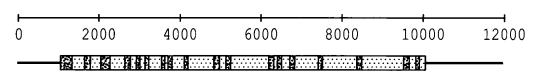


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#### BRANCHING ENZYME-II GENES

#### INTRON/EXON STRUCTURE OF WHEAT BE-II



SCHEMATIC DIAGRAM OF A cDNA BE-II

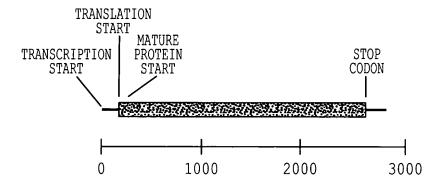


FIG. 14



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# WHEAT DNA PROBED WITH THE 5' CONSERVED SEQUENCE OF SBE II.

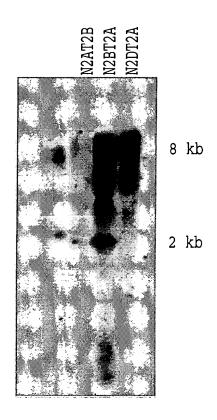


FIG. 15



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## COMPARISON OF N-TERMINAL SEQUENCES OF SOLUBLE STARCH SYNTHASE

GRYVAELSREGPAARP

DEDUCED FROM WHEAT cDNA

**GPYVAELSPEGPAAPP** 

WHEAT N-TERMINAL

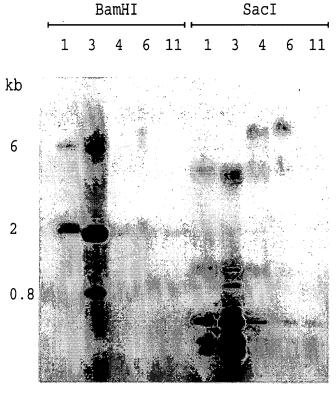
FIG. 16



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### SOLUBLE STARCH SYNTHASE GENOMIC CLONES



PROBED WITH SM-2 FULL LENGTH cDNA

FIG. 17

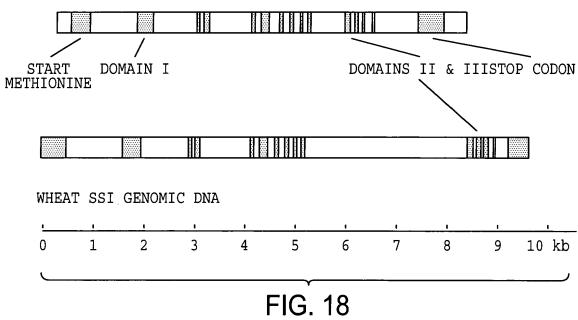


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#### INTRON EXON STRUCTURE - WHEAT SSI

RICE SSI GENOMIC DNA





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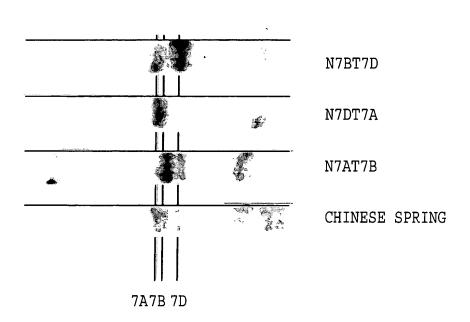


FIG. 19



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	80	ATACTACATACTATATGCTTGCACCCAAGGGACACTTTTATAACTATTCTGGCTGTGGGA +									
a b c		T T Y Y M L A P K G H F Y N Y S G C G N - I L H T I C L H P R D T F I T I L A V G - Y Y I L Y A C T Q G T L L * L F W L W E -									
	140	ATACCTTCAACTGTAATCATCCTGTGGTTCGTCAATTCATTGTAGATTGTTTAAGATACT ++									
a b c		T F N C N H P V V R Q F I V D C L R Y W - I P S T V I I L W F V N S L * I V * D T - Y L Q L * S S C G S S I H C R L F K I L -									
	200	GGGTGACGGAAATGCATGTTGATGGTTTTCGTTTTGACCTT ++ 240 CCCACTGCCTTTACGTACAACTACCAAAAGCAAAACTGGAA									
a b c		VTEMHVDGFRFDL - G*RKCMLMVFVLT - GDGNAC*WFSF*P -									
EN	NZYME	S THAT DO CUT:									
	NONE	}									
EN	1ZYME	S THAT DO NOT CUT:									
	EcoR	I									

FIG. 20A





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# COMPARISON OF WHEAT DEBRANCHING ENZYME-I (WDBE-I) PCR FRAGMENT WITH MAIZE SUGARY-1 DNA SEQUENCE

SUGARY.DNA WHEAT1.DNA		1107 GTGATCATGO          GTGATCATGO 6		1127 TCAATCATACA           TCAACCATACA 26		
FILE NAME SUGARY.DNA WHEAT1.DNA	1158 ATTA:      ATTA: 57			1187 ATAGTACATA(           ATACTACATA( 86		
FILE NAME SUGARY.DNA WHEAT1.DNA				1247 ATACCTTCAA          ATACCTTCAA 146		
FILE NAME SUGARY.DNA WHEAT1.DNA		1287 GTGGATTGCT 		1307 GGGTAACAGAA            GGGTGACGGAA 206	1327 GATGGTTTTC           GNTGGTTTTC 226	1337. GTTTTGA         GTTTTGA 236
FILE NAME SUGARY.DNA WHEAT1.DNA		1347 GCATCTATAC         GCATCTNC 246	1357 TT-G   TTTNAAA 256			
	RCENTAGE WINDOW MENT WINI	84% OOW 86%	(219/260 (219/253			

FIG. 20B



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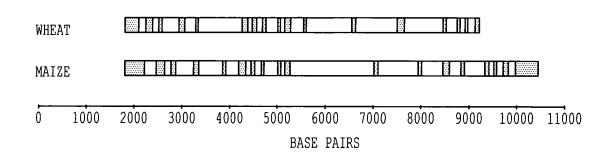


FIG. 20C

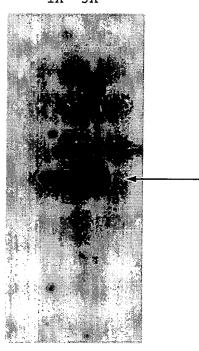


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## SOUTHERN BLOT OF T. tauschii GENOMIC DNA

1X 3X



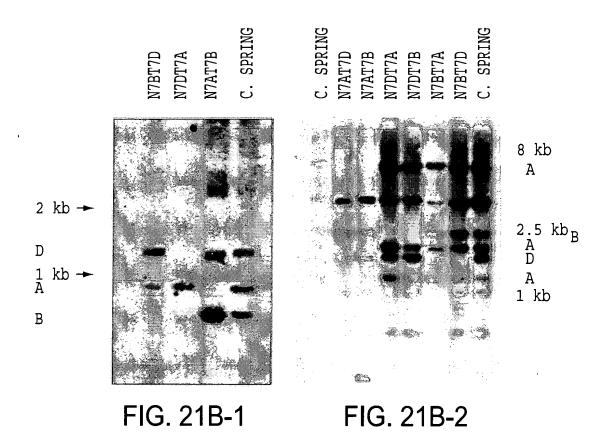
BamHI DIGEST

 $T.\ tauschii$  GENOMIC DNA PROBED WITH THE WHEAT DEBRANCHING ENZYME PCR PRODUCT

FIG. 21A



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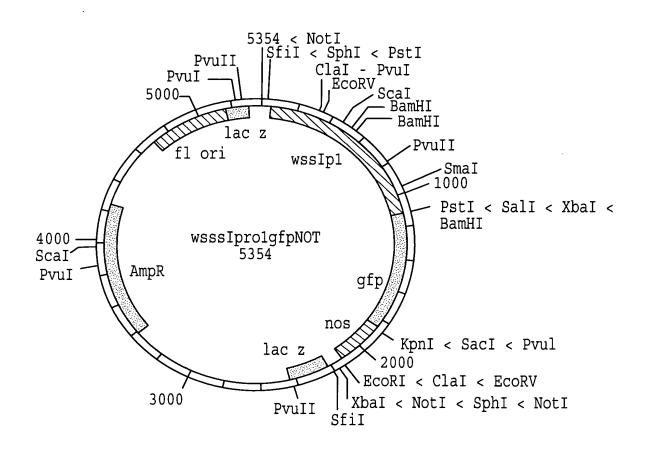


FIG. 22A



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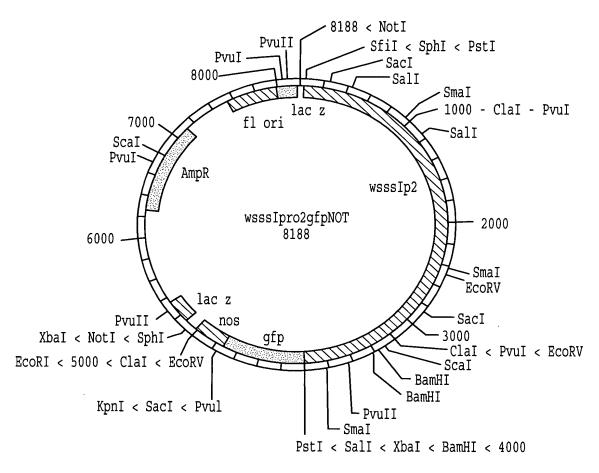


FIG. 22B



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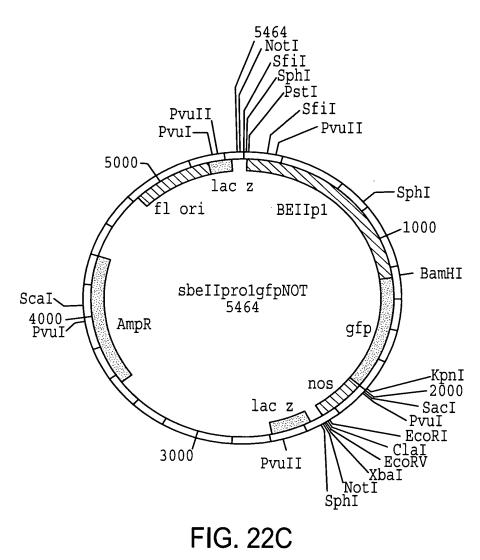


FIG. 22C



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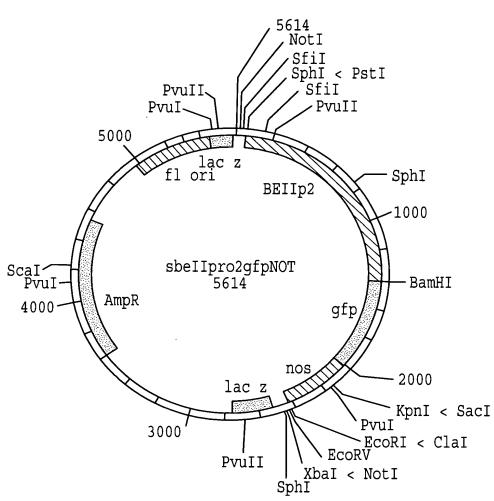


FIG. 22D



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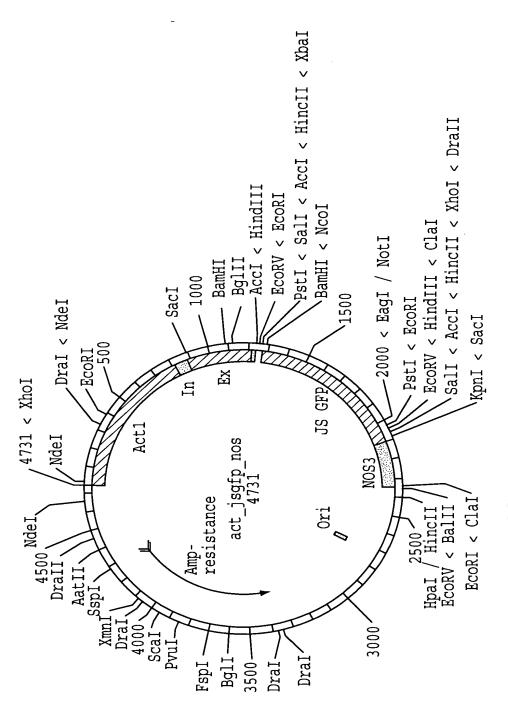


FIG. 22E



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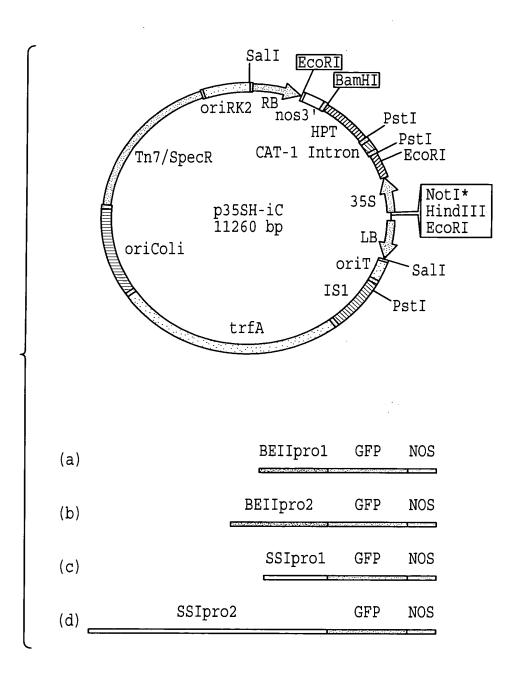


FIG. 23



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